



REC'D PCT 01 FEB 2002 #1  
09/743690

SEQUENCE LISTING

<110> Christeller, John Tane  
Sutherland, Paul William  
Murray, Colleen  
Markwick, Ngaire Patricia  
Philip, Bruce Allan  
Malone, Louise Anne  
Burgess, Elisabeth Phyllis  
Phung, Margaret Mary  
Phung, Thai Hong  
The Horticulture and Food Research Institute of  
New Zealand Limited

<120> Chimeric Polypeptides Allowing Expression of  
Plant-Noxious Proteins

<130> 020829-000100US

<140> US 09/743,690  
<141> 2001-01-12

<150> NZ 331002  
<151> 1998-07-15

<150> WO PCT/NZ99/00110  
<151> 1999-07-15

<160> 18

<170> PatentIn Ver. 2.1

<210> 1  
<211> 324  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:potato  
proteinase inhibitor I (PPI-I/pUC19)

<220>  
<221> CDS  
<222> (1)..(324)  
<223> PPI-I/pUC19

<400> 1  
atggagtc aa agtttgctca catcattgtt ttctttcttc ttgcaacttc ctggaaact 60  
ctcttggcac gaaaagaaag tggatggacca gagatcttag aacttcaaaa ggaatttggaa 120  
tgcaatggaa aacaaagggt gccagaactt attgggtac caacaaagct tgctaagggg 180  
ataattgaga aggaaaattc actcataact aatgttcaga tactactgaa tggttctcca 240  
gtcacaatgg attatcggtt taatcgagtt cgtcttttg ataacatggg ggggtgatgtt 300  
gtacaaattc cttaggggtggc tttaa 324

<210> 2  
<211> 576  
<212> DNA  
<213> Artificial Sequence

```

<220>
<223> Description of Artificial Sequence:avidin cDNA
      (pGEMav)

<220>
<221> CDS
<222> (44)..(502)
<223> avidin (pGEMav)

<220>
<221> sig_peptide
<222> (44)..(115)
<223> signal sequence

<400> 2
gaattccgca aggagcacac ccggctgtcc acctgctgca gagatggtgc acgcaacctc 60
cccgctgctg ctgctgctgc tgctcagcct ggctctgggt gctcccccggta tccctgccag 120
aaagtgcctcg ctgactggga aatggaccaa cgatctgggc tccaaatcata ccatcggggc 180
tgtgaacagc agaggtgaat tcacaggcac ctacatcaca gccgttaacag ccacatcaaa 240
tgagatcaaa gagtcaccac tgcattggac acaaaacacc atcaacaaga ggacccagcc 300
cacctttggc ttcaccgtca attggaaattt ttcagagtcc accactgtct tcacgggcca 360
gtgcttcata gacaggaatg ggaaggaggt cctgaagacc atgtggctgc tgcggtcaag 420
tggtaatgac attggtgatg actggaaagc taccagggtc ggcataaca tcttcactcg 480
cctgcgcaca cagaaggagt gaggatggcc ccgcaaaagcc agcaacaatg ccggagtgct 540
gacactgctt gtgatattcc tccccaaataa agctt 576

<210> 3
<211> 401
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:streptavidin
      cDNA (Streptavidin/pUC19)

<220>
<221> CDS
<222> (11)..(400)
<223> streptavidin (Streptavidin/pUC19)

<400> 3
gaattccgcat atggctgaag ctggtatcac cggtaacttgg tacaaccagc tggggtctac 60
cttcatcggtt accgctgggt ctgacgggtc actgaccggg acttacgaaa gcgctgttgg 120
taacgctgaa agccgttatg ttctgaccgg tcgttacgac tctgctccgg ctaccgacgg 180
ttctggtaact gctctgggtt ggaccgttgc ttggaaaaac aactaccgtt acgctcactc 240
tgctaccacc tggctctggcc agtacgttgg tggctgtgaa gctcgtatca acacccagtg 300
gctgctgacc tctggtacca ccgaagctaa cgcttggaaa tctaccctgg ttggtcacga 360
cacgttaccc aaagttaaac cgtctgtgc ttctatctgt a 401

<210> 4
<211> 584
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:potato
      proteinase inhibitor II (PPI-II/pUC19)

```

```

<220>
<221> CDS
<222> (1)..(584)
<223> PPI-II/pUC19

<220>
<221> sig_peptide
<222> (1)..(212)
<223> signal sequence

<400> 4
atggatgttc acaaggaagt taatttcgtt gcttacctac taattgttct tggtaagatt 60
ttcccttact cctttgtttt aaaaaataaa aaaacaaaaa aatcttggt ttatacatat 120
atatacacac aagtagtttt attttttcc ttatattat atttgttgc ggaatatttc 180
tacttgcgtt tag cgtgggtggaa catgttgcgt cgaagatctg tactaaagaa tggtaatc 240
ttgggttgg gatatgccca cgttcagaag gaagtccgaa aaatccata tgcataatt 300
gttgcgtcagg ctataagggt tgtaattatt atagtgttt cgggagattt atttgcgaag 360
gagaatctga cctaaaaaac cccaaagctt gcccctaaa ttgtgatata aatattgcct 420
attcaagatg cccccattca gaaggaaaat cgctaattt tcccacccgaa tgtaccacat 480
gttgcacagg gtacaagggt tgctactatt tcgtaaaaa tggcaagttt gtatgcgaag 540
gagagagtga tgaacccaaag gcaaatatgt accctgcaat gtga 584

<210> 5
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:altered Bam H I
      site

<400> 5
ggagatccaa ccatg 15

<210> 6
<211> 486
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PPI-I/Avidin
      gene fusion

<220>
<221> CDS
<222> (1)..(486)
<223> PPI-I/Avidin fusion protein

<400> 6
atggagtcaa agtttgctca catcattgtt ttctttcttc ttgcaactcc ctttgaaact 60
ctcttggcac gaaaagaaag tgatggacca gagatccctg ccagaaagtg ctcgctgact 120
ggaaatgga ccaacgatct gggctccaaat atgaccatcg gggctgtgaa cagcagaggt 180
gaattcacag gcacctacat cacagccgtt acagccacat caaatgagat caaagagtca 240
ccattgcattt ggacacaaaaa caccatcaac aagaggaccc agcccacctt tggcttcacc 300
gtcaattgga agtttcaga gtccaccaact gtcttcacgg gccagtgcattt catagacagg 360
aatggaaagg aggtcctgaa gaccatgtgg ctgctgcgtt caagtgtttaa tgacattgg 420
gtgactgga aagctaccag ggtcggcatc aacatcttca ctcgcctgcg cacacagaag 480
gagtga 486

```

<210> 7  
 <211> 161  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:PPI-I/Avidin  
 fusion protein

<400> 7  
 Met Glu Ser Lys Phe Ala His Ile Ile Val Phe Phe Leu Leu Ala Thr  
 1 5 10 15  
 Pro Phe Glu Thr Leu Leu Ala Arg Lys Glu Ser Asp Gly Pro Glu Ile  
 20 25 30  
 Pro Ala Arg Lys Cys Ser Leu Thr Gly Lys Trp Thr Asn Asp Leu Gly  
 35 40 45  
 Ser Asn Met Thr Ile Gly Ala Val Asn Ser Arg Gly Glu Phe Thr Gly  
 50 55 60  
 Thr Tyr Ile Thr Ala Val Thr Ala Thr Ser Asn Glu Ile Lys Glu Ser  
 65 70 75 80  
 Pro Leu His Gly Thr Gln Asn Thr Ile Asn Lys Arg Thr Gln Pro Thr  
 85 90 95  
 Phe Gly Phe Thr Val Asn Trp Lys Phe Ser Glu Ser Thr Thr Val Phe  
 100 105 110  
 Thr Gly Gln Cys Phe Ile Asp Arg Asn Gly Lys Glu Val Leu Lys Thr  
 115 120 125  
 Met Trp Leu Leu Arg Ser Ser Val Asn Asp Ile Gly Asp Asp Trp Lys  
 130 135 140  
 Ala Thr Arg Val Gly Ile Asn Ile Phe Thr Arg Leu Arg Thr Gln Lys  
 145 150 155 160  
 Glu

<210> 8  
 <211> 626  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial  
 Sequence:PPI-II/Streptavidin gene fusion

<220>  
 <221> CDS  
 <222> (1)...(626)  
 <223> PPI-II/Streptavidin fusion protein

<220>  
 <221> modified\_base  
 <222> (585)  
 <223> n = g, a, c or t

<400> 8  
 atggatgttc acaaggaagt taatttcgtt gcttacctac taattgttct tggtaagatt 60  
 ttccttact cctttgtttt aaaaaataaa aaaaacaaaaaa aaatcttggt ttatacatat 120  
 atatacacac aagtagtttt attttttcc tttatattat atttgttcta ggaatatttc 180  
 tacttgttag cgtgggtggaa catgttgatg cgaagatctg tactaagaat tcgcatatgg 240  
 ctgaagctgg tattcacccgt acttggtaca accagctggg gtctaccttc atcgttaccg 300  
 ctgggtctga cggtgcaactg accggtaactt acgaaagcgc tggtggtaac gctgaaagcc 360  
 gttatgttct gaccggtcgt tacgactctg ctccggctac cgacggttct ggtactgctc 420  
 tgggttggac cgttgcttgg aaaaacaact accgtaacgc tcactctgct accacctgt 480

ctggccagta cgttgggtggt gctgaagctc gatatcaacac ccagtggctg ctgacacctg 540  
 gtaccaccca agctaaccgtc tggaaatcta ccctgggttgg tcacnacacg ttcacccaaag 600  
 ttaaaccgtc tgctgcttct atctag 626

<210> 9  
 <211> 168  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial  
 Sequence:PPI-II/Streptavidin fusion protein

<400> 9  
 Met Asp Val His Lys Glu Val Asn Phe Val Ala Tyr Leu Leu Ile Val  
 1 5 10 15  
 Leu Gly Ile Phe Leu Leu Val Ser Val Val Glu His Val Asp Ala Lys  
 20 25 30  
 Ile Cys Thr Lys Asn Ser His Met Ala Glu Ala Gly Ile Thr Gly Thr  
 35 40 45  
 Trp Tyr Asn Gln Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala Asp  
 50 55 60  
 Gly Ala Leu Thr Gly Thr Tyr Glu Ser Ala Val Gly Asn Ala Glu Ser  
 65 70 75 80  
 Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser Ala Pro Ala Thr Asp Gly  
 85 90 95  
 Ser Gly Thr Ala Leu Gly Trp Thr Val Ala Trp Lys Asn Asn Tyr Arg  
 100 105 110  
 Asn Ala His Ser Ala Thr Thr Trp Ser Gly Gln Tyr Val Gly Gly Ala  
 115 120 125  
 Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu Thr Ser Gly Thr Thr Glu  
 130 135 140  
 Ala Asn Ala Trp Lys Ser Thr Leu Val Gly His Asp Thr Phe Thr Lys  
 145 150 155 160  
 Val Lys Pro Ser Ala Ala Ser Ile  
 165

<210> 10  
 <211> 638  
 <212> DNA  
 <213> Streptomyces avidinii

<220>  
 <221> CDS  
 <222> (50)..(601)  
 <223> streptavidin

<220>  
 <221> sig\_peptide  
 <222> (50)..(121)  
 <223> signal sequence

<400> 10  
 ccctccgtcc ccgccccggca acaacttaggg agtatttttc gtgtctcac atg cgc aag 58  
 Met Arg Lys  
 1

atc gtc gtt gca gcc atc gcc gtt tcc ctg acc acg gtc tcg att acg	106
Ile Val Val Ala Ala Ile Ala Val Ser Leu Thr Thr Val Ser Ile Thr	
5 10 15	
gcc agc gct tcg gca gac ccc tcc aag gac tcg aag gcc cag gtc tcg	154
Ala Ser Ala Ser Ala Asp Pro Ser Lys Asp Ser Lys Ala Gln Val Ser	
20 25 30 35	
gcc gcc gag gcc ggc atc acc ggc acc tgg tac aac cag ctc ggc tcg	202
Ala Ala Glu Ala Gly Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Ser	
40 45 50	
acc ttc atc gtg acc gcg ggc gcc gac ggc gcc ctg acc gga acc tac	250
Thr Phe Ile Val Thr Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr	
55 60 65	
gag tcg gcc gtc ggc aac gcc gag agc cgc tac gtc ctg acc ggt cgt	298
Glu Ser Ala Val Gly Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg	
70 75 80	
tac gac agc gcc ccg gcc acc gac ggc agc ggc acc gcc ctc ggt tgg	346
Tyr Asp Ser Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp	
85 90 95	
acg gtg gcc tgg aag aat aac tac cgc aac gcc cac tcc gcg acc acg	394
Thr Val Ala Trp Lys Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr	
100 105 110 115	
tgg agc ggc cag tac gtc ggc ggc gcc gag gcg agg atc aac acc cag	442
Trp Ser Gly Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln	
120 125 130	
tgg ctg ctg acc tcc ggc acc acc gag gcc aac gcc tgg aag tcc acg	490
Trp Leu Leu Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr	
135 140 145	
ctg gtc ggc cac gac acc ttc acc aag gtg aag ccg tcc gcc gcc tcc	538
Leu Val Gly His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Ser	
150 155 160	
atc gac gcg gcg aag aag gcc ggc gtc aac aac ggc aac ccg ctc gac	586
Ile Asp Ala Ala Lys Lys Ala Gly Val Asn Asn Gly Asn Pro Leu Asp	
165 170 175	
gcc gtt cag cag tag tcgcgtccccg gcaccggcggtgccccggac ctcggcc	638
Ala Val Gln Gln	
180	

<210> 11  
 <211> 183  
 <212> PRT  
 <213> *Streptomyces avidinii*

<400> 11  
 Met Arg Lys Ile Val Val Ala Ala Ile Ala Val Ser Leu Thr Thr Val  
 1 5 10 15  
 Ser Ile Thr Ala Ser Ala Ser Ala Asp Pro Ser Lys Asp Ser Lys Ala  
 20 25 30  
 Gln Val Ser Ala Ala Glu Ala Gly Ile Thr Gly Thr Trp Tyr Asn Gln  
 35 40 45

Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala Asp Gly Ala Leu Thr  
 50 55 60  
 Gly Thr Tyr Glu Ser Ala Val Gly Asn Ala Glu Ser Arg Tyr Val Leu  
 65 70 75 80  
 Thr Gly Arg Tyr Asp Ser Ala Pro Ala Thr Asp Gly Ser Gly Thr Ala  
 85 90 95  
 Leu Gly Trp Thr Val Ala Trp Lys Asn Asn Tyr Arg Asn Ala His Ser  
 100 105 110  
 Ala Thr Thr Trp Ser Gly Gln Tyr Val Gly Gly Ala Glu Ala Arg Ile  
 115 120 125  
 Asn Thr Gln Trp Leu Leu Thr Ser Gly Thr Thr Glu Ala Asn Ala Trp  
 130 135 140  
 Lys Ser Thr Leu Val Gly His Asp Thr Phe Thr Lys Val Lys Pro Ser  
 145 150 155 160  
 Ala Ala Ser Ile Asp Ala Ala Lys Lys Ala Gly Val Asn Asn Gly Asn  
 165 170 175  
 Pro Leu Asp Ala Val Gln Gln  
 180

<210> 12  
 <211> 22  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:forward M13  
 (lacZ) primer [Perkin Elmer]

<400> 12  
 gccagggttt tcccaagtac ga

22

<210> 13  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:reverse M13  
 (lacZ) primer [Perkin Elmer]

<400> 13  
 gagcggataa caatccaca cagg

24

<210> 14  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:avidin upstream  
 primer

<400> 14  
 gcacacccgg ctgtccacct g

21

<210> 15  
 <211> 22  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: PPI-I  
 phosphorylated mutagenic primer

<220>  
 <221> modified\_base  
 <222> (1)  
 <223> n = 5' phosphorylated g

<400> 15  
 natggaccag agatcttaga ac

22

<210> 16  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: avidin  
 phosphorylated mutagenic primer

<220>  
 <221> modified\_base  
 <222> (1)  
 <223> n = 5' phosphorylated g

<400> 16  
 ngctcccgaa atccctgcca g

21

<210> 17  
 <211> 21  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR sense  
 primer

<400> 17  
 ctgcaggatcg actctagagg a

21

<210> 18  
 <211> 27  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR antisense  
 primer

<400> 18  
 ggtgaattct tagtacagat cttcgca

27